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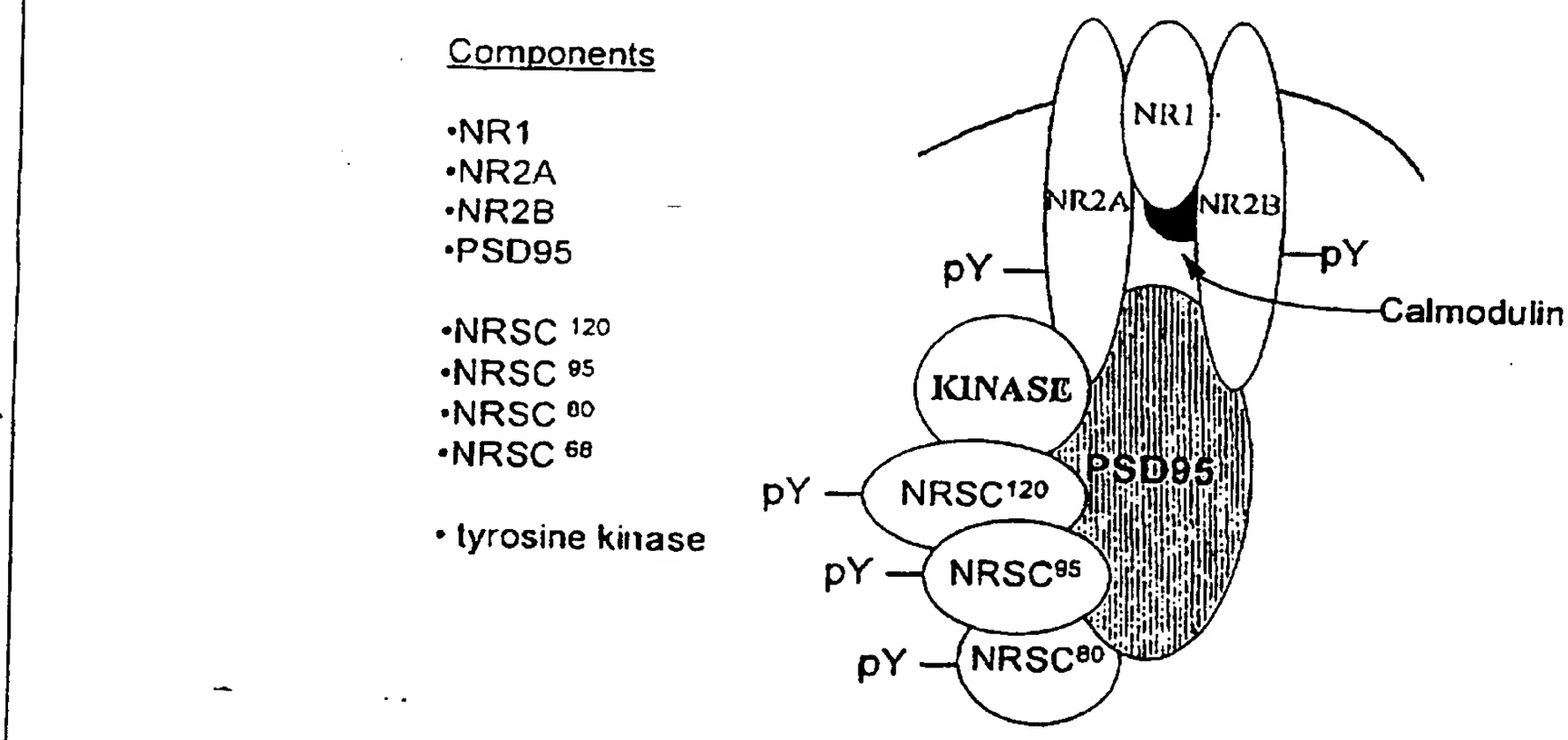

(57) Abstract

The structure of an NMDA receptor signalling complex (NRSC) is elucidated, and a method disclosed for determining whether a candidate substance is likely to be effective in modifying the function of an NMDA or non-NMDA glutamate neurotransmitter receptor, the method comprising the step of monitoring the effect of the substance on the activity or signalling capacity of the NRSC, or a component thereof. Preferably the complex or component comprises at least the Post-Synaptic Density 95 (PSD95) protein and a tyrosine kinase, together, optionally, with one or more additional tyrosine phosphorylated proteins.

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NEUROTRANSMITTERS

Field of the Invention

This invention relates to methods of identifying substances which are capable of modifying the function of neurotransmitters, in particular NMDA and non-NMDA glutamate neurotransmitters.

Background of the Invention

The NMDA receptor is an important neurotransmitter receptor found at most synapses in the brain. The activation of this receptor by the neurotransmitter glutamate produces biochemical changes at the synapse. The effects of activation of the receptor and its biochemical pathway produce profound effects on the function of synapses and thereby change behaviour. The biochemical pathway from the NMDA receptor is likely to be fundamental to an understanding of stroke pathology as well as to normal physiological events including learning and memory. There is a demand for drugs that interfere with signalling by the NMDA receptor. The main use of NMDA receptor inhibitors would be to prevent the spread, and limit the brain damage, occurring with stroke. The obvious targets for these drugs have been the NMDA receptor itself, and a number of such drugs have been developed and are undergoing clinical trial. Another beneficial application of such drugs is likely to be cognitive enhancement.

The applicants have identified and purified several brain proteins that bind to the NMDA receptor and which are components of the NMDA receptor signalling pathway. The multiprotein complex formed by these proteins is referred to in this specification as the "NRSC" (derived from NMDA Receptor Signalling Complex). A proposed schematic structure for the NRSC is presented at Figure 1.

The identification of the primary components of the NRSC, effectively provides a method whereby putative drugs can be readily screened for efficacy, and it is envisaged that the method will be suitable not only for drugs useful in interfering with the function of the NMDA receptor, but also more broadly for any glutamate transmitter. Furthermore, the NRSC is also likely to prove an attractive target for such drugs and may provide special advantages over the simple NMDA receptor blockers. For example, it may be possible to develop drugs that interfere with the assembly and composition of the NRSC in such a way as to inhibit or enhance signalling from the NMDA receptor.

Summary of the Invention

In broad terms, the invention provides a method of determining whether a candidate substance is likely to be effective in modifying the function of an NMDA or non-NMDA glutamate neurotransmitter receptor, the method comprising the step of monitoring the effect of the substance of the activity or signalling capacity of an NRSC protein complex (as defined herein), or on a component of a said complex. Preferably, the complex or component comprises at least one, or any combination, of the following:

(a) an NMDA receptor ion channel subunit (such as NR1, NR2A, or NR2B);

(b) PSD95, or a protein homologous thereto (such as Chapsyn-110);

(c) a tyrosine kinase (such as Fyn or Src); and

(d) a tyrosine phosphatase,

together, optionally, with one or more additional tyrosine phosphorylated proteins.

The protein complex may be an NRSC complex isolated from brian tissue, or may be artificially constructed.

The principal application of the invention is likely to be in the identification of drugs for treating humans, in which case a natural or artificial human NRSC complex will be used. However, the invention may also fined use in the identification of drugs for treating other animals, in particular mammals.

Brief description of the Figures

The invention is hereinafter described in more detail by way of example only, with reference to the accompanying figures, in which:-

Figure 1 is a schematic proposed structure for the NRSC;

Figure 2 is an electrophoresis blot of immunoprecipitated NMDA-R1 subunits from mouse forebrain NMDA receptors;

Figure 3 is an electrophoresis blot of NMDA NR1 subunits, immunoprecipitated with antibodies specific to NR2A and NR2B subunits; and

Figure 4 is an electrophoresis blot of PSD95, immunoblotted with antiphosphotyrosine antibodies.

Detailed description

The NMDA subtype of glutamate receptor has been implicated in a wide variety of important physiological and pathological phenomena in the brain. The most extensively studied example of NMDA receptor dependent signalling is found in experiments on long-term potentiation (LTP) in CA3-CA1 synapses in the rodent hippocampus. In this model system, the activation of the NMDA receptor leads to an enhancement of synaptic transmission at the activated synapse. Evidence has emerged for a plethora of physiological mechanisms that may contribute to the change in synaptic efficacy, including changes in post-synaptic transmitter receptor sensitivity, activation of silent receptors, generation of retrograde messengers to the pre-synaptic terminal, structural changes in dendritic spines, signal transduction of local protein translation machinery

and activation of transcription in the nucleus. To understand the generation of these complex and diverse cellular responses it is necessary to determine the molecular events proximal to the NMDA receptor.

The NMDA receptor in forebrain structures, including the CA1 pyramidal cells of the hippocampus is a heteromeric complex of three channel-forming membrane proteins designated as NR1, NR2A and NR2B. The homomultimer of NR1 subunits is sufficient to form the ion-channel/receptor whereas the NR2 subunits modify the properties of NR1 to produce the heteromultimeric mature channel. The activation of the NMDA receptor requires binding of glutamate as well as membrane depolarisation, which leads to opening of the pore and influx of extracellular calcium. The calcium influx is necessary but not sufficient for the expression of LTP, since calcium influx to the dendritic spine through other ion-channels does not support the same form of LTP induced by NMDA receptors. This suggests that the NMDA receptor does more than regulate calcium influx and may activate signal transduction cascades, perhaps by direct interaction with cytoplasmic signalling proteins that can contribute to synaptic plasticity.

The first studies aimed at identifying components of an NMDA receptor signal transduction pathway utilized pharmacological inhibitors introduced into the post-synaptic neuron. Although these experiments implicated serine-threonine and tyrosine kinases in the induction of LTP, the inhibitors lack the specificity either to implicate a specific kinase or to define the perturbed step in the signalling pathway. The introduction of knockout mice provided a more specific tool for incrimination of a specific protein and opened the possibility of combining genetic and biochemical tools to dissect the post-synaptic signalling underlying LTP.

The applicants have focused on identifying the role of the non-receptor tyrosine kinases in LTP. Tyrosine kinase

inhibitors block the induction of LTP when introduced into the post-synaptic cell (O'Dell et al, 1991), and can be phenocopied by mice carrying mutations in the *fyn* gene (Grant et al, 1992). Fyn is a member of the Src family of tyrosine kinases and comparison of *fyn*, *src* and *yes* mutants indicates that Fyn has a specific signalling role in LTP (Grant et al, 1992; Grant et al, 1995). Together with data showing that the injection of Src family kinases can modulate NMDA receptor currants, this suggests that NMDA receptor signal transduction may require the interaction with Fyn or related kinases.

To explore the role of tyrosine kinases in NMDA receptor signal transduction the applicants isolated the NMDA receptor and associated proteins from the brain. It was found that the NMDA receptor is associated with multiple proteins, some of which are tyrosine phosphorylated. This NMDA receptor complex contains the Post-Synaptic Density 95 (PSD95) protein, which appears to be required for the assembly and tyrosine phosphorylation of the complex. The NMDA receptor was found to exist in two forms, either bound to PSD95 or free from PSD95. Using mutant mice it was found that the ratio of these two forms of the NMDA receptor is regulated by Fyn. In addition, Fyn was required for the phosphorylation of the NMDA receptor and other components of the complex, which indicates that phosphorylation modulates the assembly of the complex. The assembly and tyrosine phosphorylation of the NMDA receptor into the complex was also regulated during postnatal development of the brain. The NMDA receptor complex identified in this study provides a new path into the study of mechanisms of NMDA receptor signal transduction and the association of the NMDA receptor with post-synaptic proteins.

In order to identify proteins associated with the NMDA receptor-channel subunits, intact NMDA receptors from mouse forebrains were solubilized and NMDA-R1 (NR1) subunits and associated proteins were immunoprecipitated with antibodies to NR1. To identify the tyrosine phosphoproteins

associated with NR1, the NR1 immunoprecipitate was separated on SDS-PAGE and immunoblotted with antiphosphotyrosine antibodies (Figures 2 & 4). There appears to be at least 5 tyrosine phosphoproteins which are components of the NMDA receptor complex, including two major bands observed at 180kD and 120kD, a less prominent band at 95 kD, and minor bands at 80 & 68kD. Since these proteins may be components of an NMDA Receptor Signalling Complex they are herein referred to as $NRSC^{180}$, $NRSC^{120}$, $NRSC^{95}$, $NRSC^{80}$, $NRSC^{68}$ respectively.

The presence of tyrosine phosphoproteins associated with the NMDA receptor implies that a tyrosine kinase(s) may be part of the NRSC. The hypothesis that a tyrosine kinase is associated with the NR1 immunoprecipitate was tested by performing an immunecomplex kinase reaction. The immunecomplex kinase reaction revealed three major bans: pp180, pp120, pp95. These proteins are phosphorylated on tyrosine residues since the label is resistant to alkali treatment. Therefore it is likely that the NMDA receptor is associated with the tyrosine kinase (or Kinases) that is capable of phosphorylating the NRSC components $NRSC^{180}$, $NRSC^{120}$, $NRSC^{95}$.

Previous studies showed the NMDA receptor 2A (NR2A) and 2B (NR2B) subunits were tyrosine phosphoproteins. The applicants tested whether if NR2A and NR2B were components of the NRSC by immunoblotting the NR1 immunoprecipitate with antibodies specific to NR2A and NR2B (Figure 3). Both NR2A and NR2B were detectable at ~180kD and comigrated with $NRSC^{180}$. It was confirmed that NR2A and NR2B were tyrosine phosphorylated, by immunoprecipitating NR2A and NR2B from denatured extracts, separating the proteins by SDS-PAGE, and immunoblotting with antiphosphotyrosine antibodies. In addition the reciprocal experiment was performed of immunoprecipitating with antiphosphotyrosine antibodies and immunoblotting with antibodies to NR2A and NR2B. Together these data show that $NRSC^{180}$ is comprised of NR2A and NR2B.

Since NR1 has a molecular weight of ~120kD it was next tested whether $NRSC^{120}$, the second major tyrosine phosphorylated band found in NR1 immunecomplexes, was NR1. Using three antibodies that are capable of immunoprecipitating all known isoforms of NR1, it was possible to detect the presence of tyrosine phosphorylation in NR1 immunecomplexes from denatured extracts, and the presence of NR1 in immunecomplexes with antiphosphotyrosine antibodies. Although NR1 was found to comigrate with $NRSC^{120}$ on immunoblots, it was concluded that $NRSC^{120}$ is an unidentified protein found in a complex with the NMDA receptor.

Recent studies using the yeast two-hybrid system and in vitro binding studies show the NR2 subunits of the NMDA receptor can bind to the post-synaptic density protein 95 (PSD95). It was therefore postulated that the NRSC identified by immunoprecipitation of brain extracts contains PSD95. To test this, the NMDA receptor and PSD95 were each immunoprecipitated in the conditions used to identify the NRSC, separated by SDS-PAGE, and immunoblotted with antiphosphotyrosine antibodies (Figure 4). There was a striking similarity in the pattern of associated proteins, with the major bands at 180kDa, 120kDa, 95kDa in common. To confirm that PSD95 and NMDA receptor were components of the same complex, the complex was immunoprecipitated with PSD95 and immunoblotted with antibodies to NR1, NR2A and NR2B. It was found that these three subunits of the NMDA receptor were present in the PSD95 immunecomplex, indicating that the NMDA receptor can physiologically associate with PSD95 *in-vivo*, and that PSD95 is a component of the NRSC.

To assess $NRSC^{95}$ could be identified as be PSD95, the applicants tested whether PSD95 is a tyrosine phosphoprotein (Figure 4) by immunoprecipitating PSD95 from denatured extracts and immunoblotted with antiphosphotyrosine antibodies. The reciprocal experiment of immunoprecipitating with antiphosphotyrosine antibodies and immunoblotting with antibodies to PSD95 was also carried

out. It was found that PSD95 is not tyrosine phosphorylated, which together with the data showing that it is found in the NRSC suggests that NRSC^{95} is not PSD95.

The experimental data show that the NMDA receptor is associated in a multiprotein complex in-vivo. This complex is comprises the NMDA receptor subunits NR1, NR2A and NR2B and PSD95 as well as several unidentified components including NRSC^{120}, NRSC^{95} NRSC^{80}, NRSC^{58} and a tyrosine kinase. Since it is clear that the NMDA receptor-channel complex can be formed from NR1 and NR2 subunits alone, it remains to be shown whether the other components of the NRSC have any physiological role. The applicants speculate that one role for these additional components of the NRSC is to mediate signal transduction from the NMDA receptor and contribute to the expression of NMDA receptor-dependent forms of synaptic plasticity.

Electrophysiological studies of NMDA receptor mediated synaptic plasticity have shown these forms of plasticity to mature during the first postnatal weeks of rodent life. It was reasoned that if the NRSC is required for NMDA receptor dependent plasticity, then the assembly of the NRSC may develop to maturity during the same (critical) time period. The assembly of the NRSC and the tyrosine phosphorylation of its components during postnatal development were therefore examined.

The postnatal assembly of the NRSC was investigated by examining the tyrosine phosphorylated proteins associated with PSD95 or NR1 from forebrain extracts prepared from 1 day and 1,2,3,6 and 12 week old mice (Figure 4). A dramatic maturation was observed in the assembly of the NRSC. At birth and for the first 2 weeks of postnatal life there were no detectable tyrosine phosphoproteins, but during the next week a significant increase was observed in NRSC$^{180\ (NR2A/NR2B)}$, NRSC^{120} and NRSC$^{95\ (PSD95)}$ which were fully expressed by 6 and 12 weeks of age.

To gain an insight into the mechanism for the apparent onset of assembly of the NRSC during the 3rd postnatal week of life, the binding of NR1, NR2A and NR2B associated with PSD95 was measured. This was achieved by stripping the antiphosphotyrosine immunoblot of the PSD95 immunecomplexes and sequentially immunoblotting with antibodies specific to NR1, NR2A and NR2B. It was observed that these three subunits of the NMDA receptor were first detectable in the PSD95 immunecomplex at the same time as tyrosine phosphorylation was detected in the NRSC, that is during the third postnatal week.

The postnatal assembly of PSD95 with the NMDA receptor may be regulated by the levels of expression of PSD95 with age. The level of expression in the extracts was tested by immunoblotting the extracts with antibodies to PSD95 and there was found to be a significant increase in PSD95 during the first 6 weeks of postnatal forebrain development. The levels of expression of NR1, NR2A and NR2B in the extracts were also examined.

The simplest explanation of the age-dependent assembly of PSD95 with the NMDA receptor is that PSD95 is required to be expressed at sufficient levels to bind the NMDA receptor, and these levels are first achieved at about 3 weeks' postnatal age.

These observations show that the assembly of the NRSC is critically age-dependent and specifically demonstrate that the binding of PSD95 occurs later than the expression of the NMDA receptor. Since synaptic plasticity is maturing over the same time period, it is speculated that the association of PSD95 with the NMDA receptor allows the NMDA receptor to interact with signal transduction proteins required for NMDA receptor dependent forms of synaptic plasticity. To explore this possibility it was investigated whether if the age-dependent association of NMDA receptor with PSD95 is correlated with the onset of tyrosine phosphorylation of NR2 subunits.

NR2A and NR2B subunits were immunoprecipitated with an antibody and immunoblotted with antiphosphotyrosine antibodies. It was found that Tyrosine phosphorylation of NR2 is first seen at a similar time as the binding of NMDA receptor and PSD95 occurs. This is consistent with the idea that the tyrosine kinase responsible for phosphorylating the NR2 subunits may require PSD95 to facilitate the interaction of the NMDA receptor. Another possibility is that the kinase that is required to phosphorylate the NMDA receptor may regulate the interaction of the NMDA receptor with PSD95 and thereby regulate the assembly of the NRSC. The experimental evaluation of these and other models would be greatly facilitated if the physiologically relevant NMDA receptor tyrosine kinase were known, and accordingly the applicants have sought to identify this kinase.

PSD95 comprises multiple domains, including 3 PDZ domains, an SH3 and a guanylate kinase homology region (Kennedy paper 92). The interaction with NR2 is mediated via mediated via the second PDZ domain and the role of the other domains is unknown. The dramatic increase in NRSC formation during the 3rd postnatal week is a reflection of an increase in binding of PSD95 with the NMDA receptor and is coincident with the emergence of tyrosine phosphorylation and the detection of $NRSC^{130}$. These changes may reflect the recruitment of kinase activity and $NRSC^{130}$ to the NRSC by PSD95. Therefore the domains of PSD95 that have no identified binding partners may be interacting with tyrosine kinases and $NRSC^{130}$. PSD95 could serve the role as an adaptor protein between the NMDA receptor and important signalling proteins including other components of the NRSC. Consistent with this model, PSD95 can interact via PDZ domains with other PDZ containing proteins including neuronal Nitric Oxide Synthase (Bredt 96). In addition to the components of the NRSC identified in our studies, there are also likely to be components that we have not detected. For example, calmodulin was found to bind to the NR1 subunit (Ehlers et al, 1996).

As demonstrated above, there is detectable tyrosine kinase activity in NR1 immunecomplexes that can phosphorylate $NRSC^{180\ (NR2A/NR2B)}$, $NRSC^{120}$ and $NRSC^{95\ (PSD95)}$. An attempt was made to identify the kinase by immunoblotting the NR1 immunecomplexes with antibodies to Src, Fyn, Yes, FAK, Pyk2/CAKb and Csk but no specific associated proteins were detected. The failure to detect may be a result of the low stoichiometry of association using the relatively harsh extraction conditions necessary to solubilise the NMDA receptor, rather than a lack of a relevant protein interaction. Moreover, many kinases do not form stable complexes with their substrates. A different approach was therefore made, utilising mutant mice, on the basis that Fyn tyrosine kinase is a likely candidate for the role of a kinase that can phosphorylate the NRSC. Previous studies have shown that Fyn is required for long-term potentiation and thus may be involved with an NMDA receptor signal transduction cascade (Grant et al, 1992).

To determine if Src family kinases are required *in vivo* to maintain the phosphorylation of the NRSC, immunoprecipitates of the NRSC with NR1 or PSD95 antibodies from *fyn* mutant mice were examined. The precipitated proteins were immunoblotted with antiphosphotyrosine antibodies. A significant reduction in the intensity of the tyrosine phosphorylation signal was seen in all of the tyrosine phosphorylated components of the NRSC. The same blot was stripped and reprobed with antibodies to NR2B and NR1 to show that the levels of these subunits were not altered. Although this suggests a reduction in the stoichiometry of phosphorylation of NR2B, this was confirmed by carrying out a two step analysis of wild type versus *fyn* mice. Following immunoprecipitation and dissociation of the complex, NR2A and NR2B were independently immunoprecipitated and analysed for the level of tyrosine phosphorylation. The data show reduction in signal at 180kDa on antiphosphotyrosine blots, providing strong evidence for alteration of stoichiometry of phosphorylation of NR2 subunits in the NRSC in *fyn* mice. The blot was reprobed

with NR2A and NR2B to confirm that the efficiency of immunoprecipitation was the same in wild type and *fyn* samples. NRSC phosphorylation in *src* mutant mice was also examined, and it was found that the NRSC was hypophosphorylated in *src* mice and in *fyn* mice, and that the ratios of NR1 and PSD95 were unchanged in the mutants, suggesting that the integrity of the complex in the mutants is unaltered. Thus, both Fyn and Src are required for maintaining the phosphorylation level of the NMDA receptor and associated proteins *in vivo*.

The possibility that Fyn can directly phosphorylate NR2 and the other NRSC components was explored by using the NRSC as an *in vitro* substrate for Fyn. The NRSC was isolated by immunoprecipitation was antibodies to PSD95 or NR1 from wild type mice, and kinase assays were performed with purified Fyn and [γ-^{32}P]ATP. Fyn-dependent phosphorylation was observed in NRSC$^{(NR2A/NR2B)}$ and NRSC^{120}, while phosphorylation of the other NRSC proteins was not detected. Both NR2A and NR2B were found to have incorporated label, whereas NR1 had not. Furthermore, affinity-purified NR2B could be shown to be directly phosphorylated by Fyn. To support these findings further, it was found that an E. coli expressed fusion protein comprising the carboxyl-terminus of the NR2B subunit was also efficiently phosphorylated by Fyn *in vitro*. Together, the evidence from the *fyn* mutants and *in vitro* kinase assays strongly support the model that Fyn is a tyrosine kinase that directly phosphorylates the NR2 subunits and NRSC^{120} *in vivo*. These kinase assays provide a basis for assays that can be used to screen drugs that regulate the phosphorylation of the NRSC.

The action of the kinases is counteracted by tyrosine phosphatases (PTPases). A PTPase activity was identified which acts on the NRSC proteins, and which, importantly, is itself activated by the NMDA receptor. The effects of NMDA receptor stimulation on hippocampus slice preparations as used for slice electrophysiology was examined. Protein extracts were prepared and the NRSC was immunoprecipitated

and analysed for changes in tyrosine phosphorylation. Unstimulated control slices were also extracted over the range of the timecourse. The tyrosine phosphorylation was reduced in $MRSC^{180}$ and $NRSC^{120}$ following NMDA receptor activation. The blots were reprobed with NR2B, NR1 and PSD95 antibodies. The levels of these proteins were not significantly reduced over the stimulation timecourse, indicating that the stoichiometry of phosphorylation is reduced, rather than dissociation of NR2B from the complex. Whole extract samples were also blotted with antiphosphotyrosine antibodies and overall levels of tyrosine phosphoproteins were not altered. This suggests that hypoxia or another non-specific phosphatase activation does not account for the change seen, but that the hypophosphorylation is more specific to NMDA receptor subunits and the NRSC. In addition to the reduction in phosphorylation of NR2A and NR2B, the appearance of a tyrosine phosphorylated band at 140kDa ($NRSC^{140}$), and an initial reduction and then increase in $NRSC^{95}$, following stimulation indicates a more dynamic regulation of the complex. These data show that Src family kinases and an NMDA receptor-activated PTPase both act on the NMDA receptor, as well as the associated proteins. The activity of the PTPase on the NMDA receptor is likely to be of great significance, and agents which modulate its activation or effects on NRSC proteins will be potentially valuable in regulating NMDA receptor functions.

The isolation and analysis of the NRSC can be used to assay drugs both *in vitro* and *in vivo*. Such drugs may act directly on the NMDA receptor, or an proteins in the NRSC (for example PSD95), or on enzymes that regulate the post-translational state (for example the phosphorylation state) of NRSC proteins.

In vitro assays: The NRSC may be purified as described from neuronal cells, and the purified protein complex exposed to: (i) other enzymes (for example kinases or phosphatases) and their action on the NRSC tested: or (ii)

drugs or agents (including peptides) that disrupt or alter the composition or organisation of the NRSC. The action of enzymes or chemicals on the NRSC *in vitro* may be used as an assay to identify compound which modify the effects of such agents.

In vivo assays: Neural tissue (from intact living animals, brain explants, cultured neurones) may be treated with drugs that activate the NMDA receptor (or other receptors or enzymes), and the NRSC may then be purified and its composition analysed. For example, drugs that activate the NMDA receptor produce a dephosphorylation of the NRSC proteins, and this reaction can be interfered with by chemicals. The NRSC is regulated during brain development, and drugs that alter brain development could be assayed on the NRSC.

As an example of an assay, brain tissue was stimulated with the agent N-methyl-D-aspartate (NMDA), which specifically activates the NMDA receptor. The NRSC was then purified from the brain tissue, and its phosphorylation and composition analysed. The tyrosine phosphorylation was reduced in $NRSC^{180}$ (NR2A and NR2B) and $NRSC^{120}$ following NMDA receptor activation. In addition to the reduction in phosphorylation of NR2A and NR2B, the appearance of a tyrosine phosphorylated band at 140kDa ($NRSC^{140}$) and an initial reduction and then increase in $NRSC^{95}$ following stimulation indicated a more dynamic regulation of the complex. This assay may readily be extended to screen for: (i) drugs that interfere with the effects of NMDA on the NRSC as described above; and (ii) drugs that alter the phosphorylation and composition of the NRSC by acting on other receptors or enzymes that directly or indirectly regulate the NRSC.

CLAIMS

1. A method for determining whether a candidate substance is likely to be effective in modifying the function of an NMDA or non-NMDA glutamate neurotransmitter receptor, the method comprising the step of monitoring the effect of the substance on the activity or signalling capacity of an NMDA receptor signalling complex of proteins (NRSC), or on a component thereof.

2. A method according to claim 1, wherein the NRSC comprises at least one NMDA receptor ion channel subunit.

3. A method according to claim 2, wherein the NMDA receptor ion channel subunit(s) is, or are selected from, NR1, NR2A or NR2B.

4. A method according to any of claims 1 to 3, wherein the NRSC comprises PSD95, or a protein homologous thereto.

5. A method according to claim 4, wherein the homologous protein is Chapsyn-110.

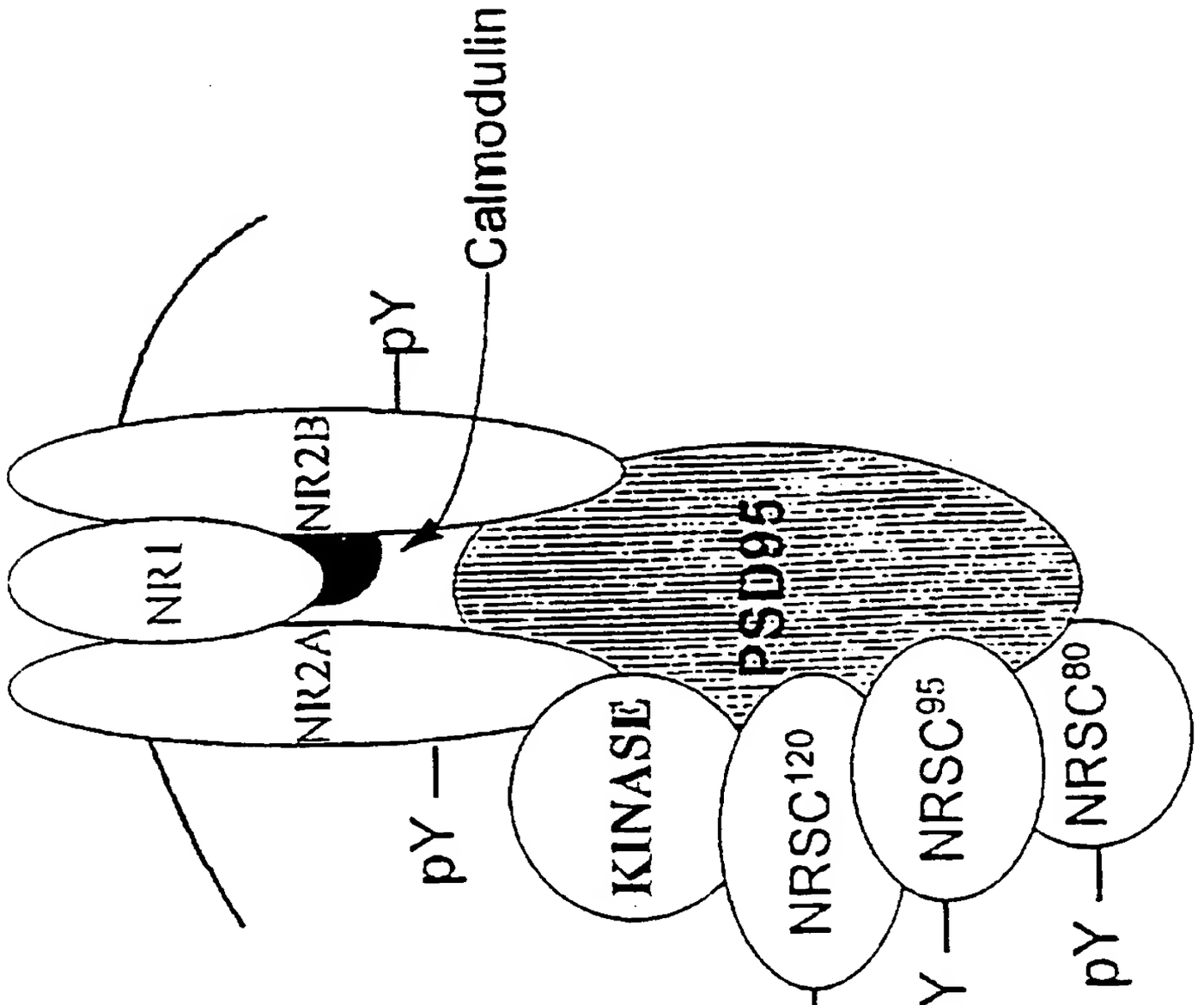
6. A method according to any preceding claim, wherein the NRSC comprises at least one tyrosine kinase.

7. A method according to claim 6, wherein the tyrosine kinase is Fyn or Src.

8. A method according to any preceding claim wherein the NRSC comprises at least one tyrosine phosphatase.

9. A method according to any preceding claim, wherein the NRSC comprises one or more additional tyrosine phosphorylated proteins.

Proposed structure of the NRSC



Components

- NR1
- NR2A
- NR2B
- PSD95
- NRSC 120
- NRSC 95
- NRSC 80
- NRSC 68
- tyrosine kinase

FIG. 1

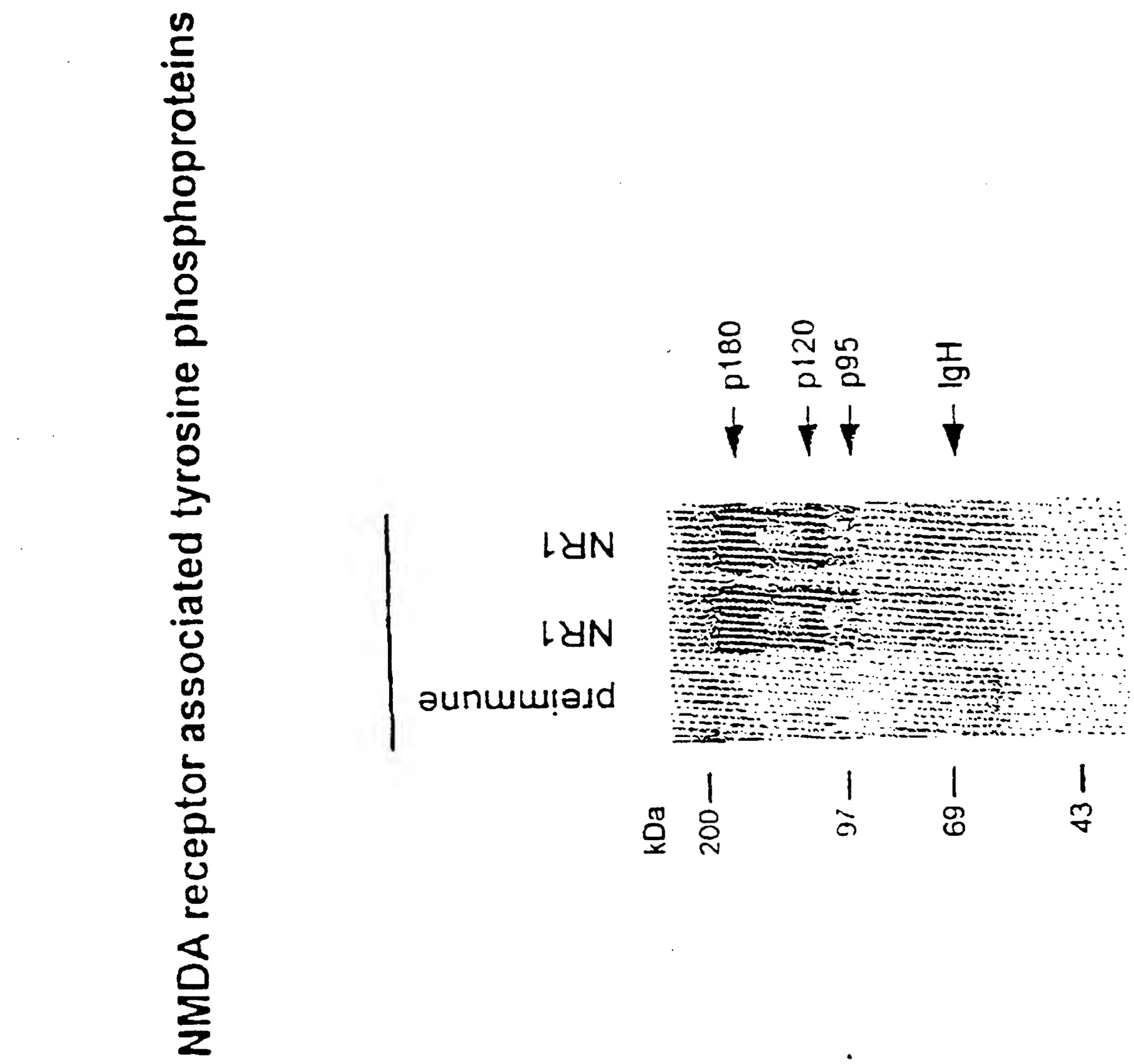


FIG. 2

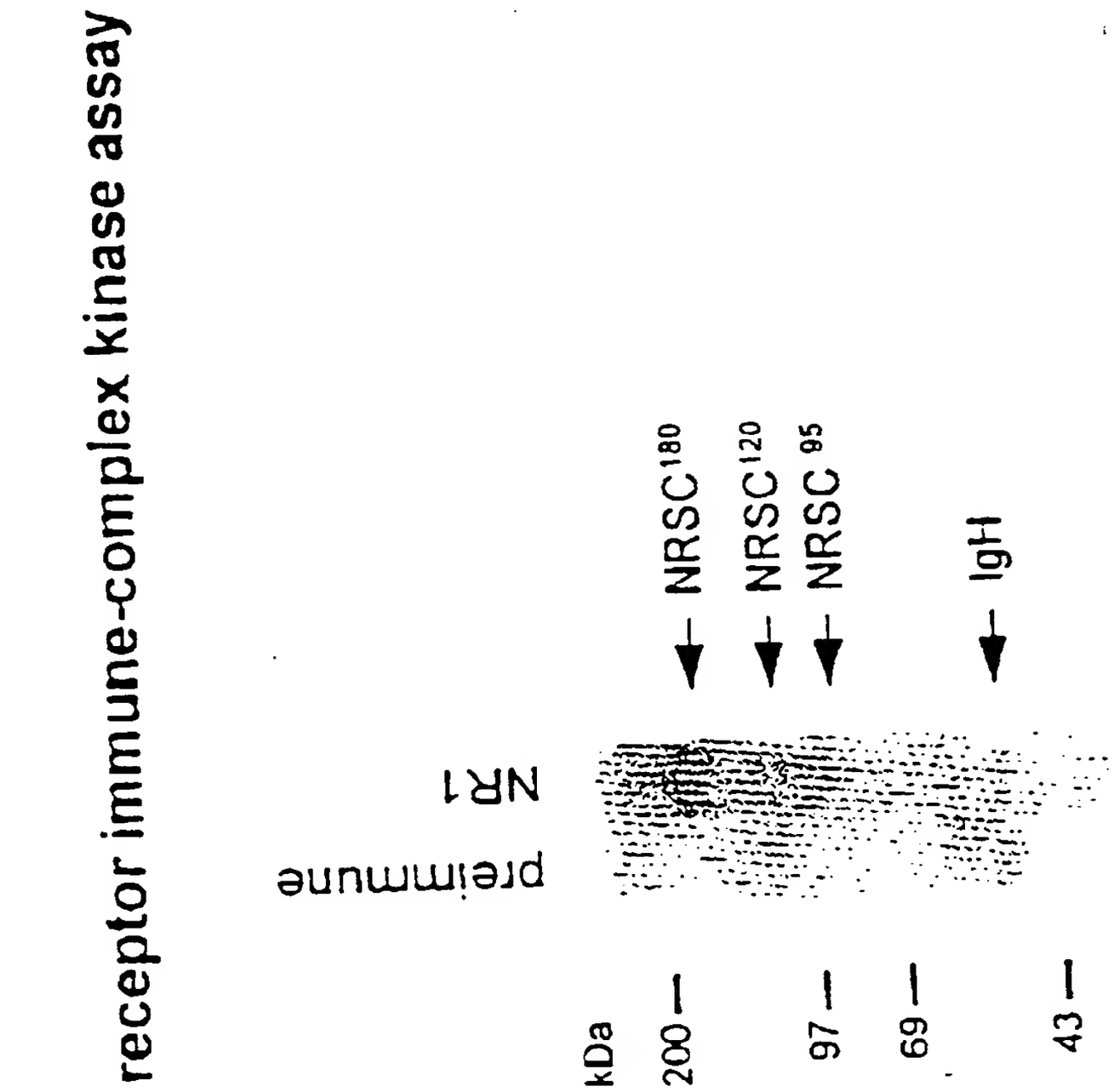


FIG. 3

The NRSC assembles with postnatal age

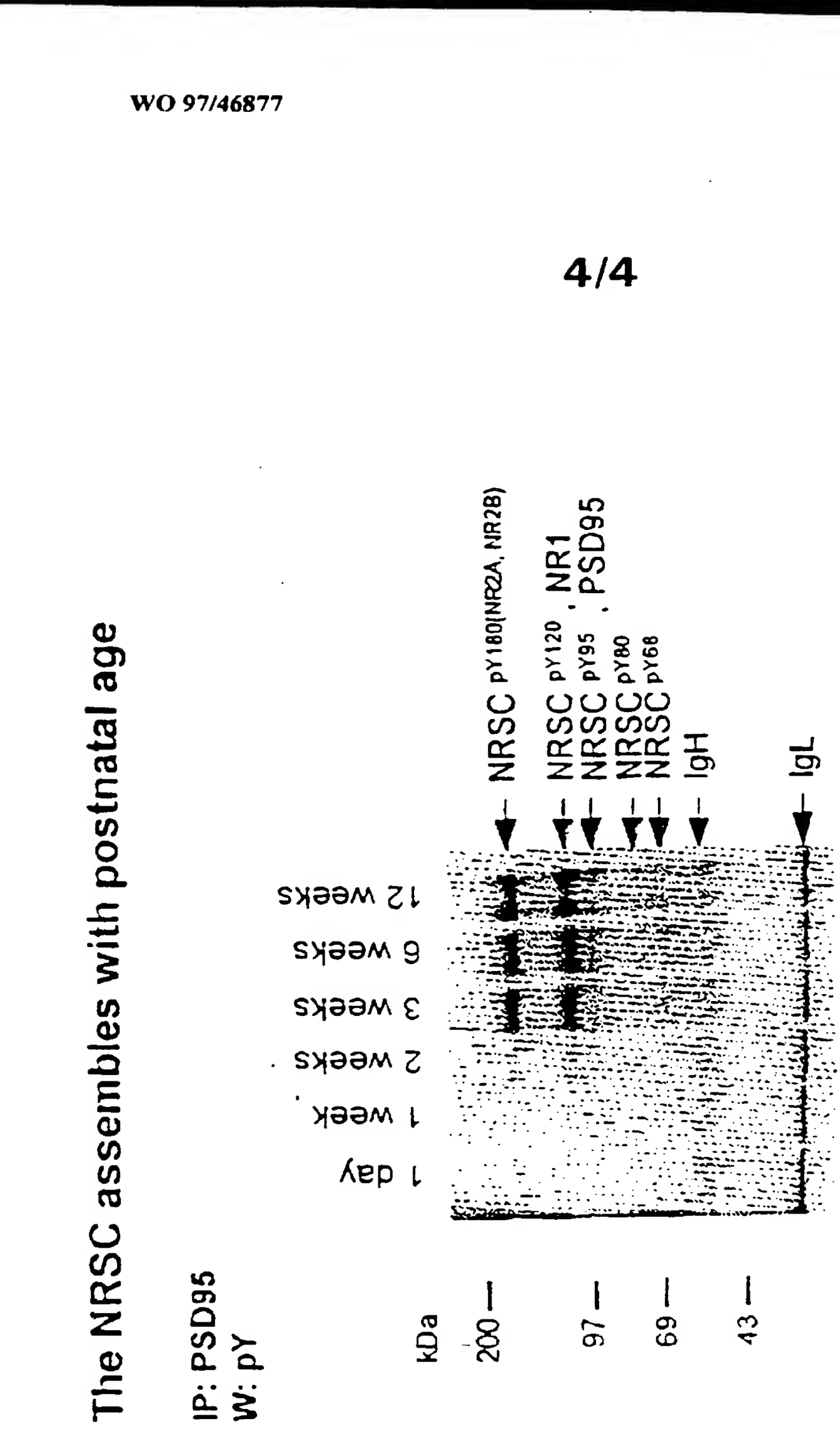


FIG. 4

INTERNATIONAL SEARCH REPORT

Intern nal Application No
PCT/GB 97/01512

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/50 C12Q1/48 C12Q1/42

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	WO 94 26301 A (KABI PHARMACIA AB ;BOURGUIGNON J P (BE)) 24 November 1994 see the whole document ---	1
X	WO 95 21612 A (NPS PHARMA INC ;MUELLER ALAN L (US); WAGENEN BRADFORD C VAN (US);) 17 August 1995 see the whole document ---	1
X	WO 91 02810 A (CHILDRENS MEDICAL CENTER) 7 March 1991 see the whole document ---	1
X	US 5 118 675 A (JIRKOVSKY IVO L ET AL) 2 June 1992 see the whole document ---	1

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X Further documents are listed in the continuation of box C.

X Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 October 1997	11. 11. 97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Moreno, C

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/GB 97/01512

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 420 155 A (KULAGOWSKI JANUSZ J ET AL) 30 May 1995 see the whole document	1
P,X	WO 96 22962 A (UNIV COLUMBIA ;NAKANISHI KOJI (US); HUANG DANWEN (US); CHOI SEOK K) 1 August 1996 see the whole document	1
P,X	T. G. SMART: "Regulation of excitatory and inhibitory neurotransmitter-gated ion channels by protein phosphorylation." CURRENT OPINION IN NEUROBIOLOGY, vol. 7, no. 3, June 1997, pages 358-367, XP002045241 see the whole document	1
P,X	S. CHEN & J. P. LEONARD: "Protein tyrosine kinase-mediated potentiation of currents from cloned NMDA receptors." JOURNAL OF NEUROCHEMISTRY, vol. 67, no. 1, July 1996, pages 194-200, XP002045242 see the whole document	1
A	M. W. SALTER ET AL: "Protein tyrosine kinase and phosphatase regulation of NMDA channels: developmental implications." RECEPTOR DYNAMICS IN NEURAL DEVELOPMENT, 1996, pages 45-58, XP002045243 see the whole document	1
A	G. KÖHR & P. H. SEEBURG: "Subtype-specific regulation of recombinantNMDA receptor-channels by protein tyrosine kinases of the scr family." JOURNAL OF PHYSIOLOGY, vol. 492, no. 2, 15 April 1996, pages 445-452, XP002045244 see the whole document	1
A	M. C. MACDONALD ET AL: "Expression of c-fos protein by M-methyl-D-aspartic acid in hypothalamus of immature female rats: blockade by MK-801 or neonatal treatment with monosodium glutamate." BRAIN RESEARCH: DEVELOPMENTAL BRAIN RESEARCH, vol. 56, no. 2, 1 November 1990, pages 294-297, XP002045245 see the whole document	1

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INTERNATIONAL SEARCH REPORT

Interr nal Application No
PCT/GB 97/01512

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	J. RODRIGUEZ ET AL: "Blockade of nitric oxide synthesis by tyrosine kinase inhibitors in neurones." NEUROPHARMACOLOGY, vol. 33, no. 11, 1994, pages 1267-1274, XP002045246 see the whole document ---	1
A	Y. T. WANG ET AL: "Ca2+-independent reduction of N-methyl-D-aspartate channel activity by protein tyrosine phosphatase." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 93, no. 4, February 1996, WASHINGTON US, pages 1721-1725, XP002045247 see the whole document -----	1

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Information on patent family members

Inter nal Application No
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